REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-18 and 21-39 are pending. To advance prosecution in this application, claim 1 is amended to specify that the modified protein claimed is:

> able to crystallize to form a crystalline monolayer and the unmodified protein is from a Lactobacillus bacterium

This amendment is supported in the specification by the paragraph bridging pages 5 and 6 and the fourth full paragraph of page 8, and also in original claim 2. Thus, they are fully supported by the original disclosure and their entry does not add new matter.

It is noted that Smit *et al.* (US 5,500,353) was cited in support of the restriction requirement. Although the restriction requirement was made final, Applicant wishes to comment on Smit *et al.* with the aim of facilitating prosecution and to help outline the differences between what is disclosed in Smit *et al.* and the pending claims.

Claim 1 refers to the unmodified S layer protein as coming from a *Lactobacillus* bacterium. In contrast, Smit *et al.* deals with an S-layer protein from a Gram-negative bacterium, *Caulobacter crescentus. Lactobacilli* are Gram-positive bacteria.

Gram-negative bacteria have different structure and properties compared to Gram-positive bacteria. In addition, Gram-positive bacteria, such as *Caulobacter crescentus* of Smit *et al.*, are found under different environmental conditions (e.g., soil, fresh water environments, waste water and effluents) while Gram-positive bacteria, and in particular *Lactobacilli,* preferentially reside in human and animal intestine and are used in various food products and beverages. The differences in where the two types of bacteria are found reflects how different are the two types of bacteria.

Not only are the bacteria used in Smit *et al.* and the *Lactobacilli* referred to by the claims completely different, but their S-layer proteins' structure and properties are entirely different. While the S-layer of *C. crescentus* is present as a hexagonal array (p6 symmetry), the S-layer of *Lactobacilli* is present as an oblique (p2 symmetry) array. Also the structure and properties of the monomeric units of the S-layer, the S-protein, of the *C. crescentus* used in Smit *et al.* and those of *Lactobacili* are very different. While the S-

protein of *C. crescentus* is an acidic protein with a pI 3.46, those of *Lactobacilli* are highly basic (pI 9.8).

Moreover, the way S-proteins are secreted and anchored to the cell surface is different in the two types of bacterium. The S-protein from *C. crescentus* is secreted by a fundamentally different mechanism compared to that of *Lactobacilli*. In particular, the secretion signal of *C. crescentus* is located at the C-terminus of the protein and is not cleaved off during transport over the cell membrane, in a Sec I type mechanism. In contrast, the secretion signal of *Lactobacilli* is located at the N-terminus of the S-protein and is cleaved off during translocation, in a Sec II type mechanism.

Thus, in comparing the *C. crescentus* bacterium of Smit *et al.* and *Lactobacilli* specified by claim 1 of the present application:

- the two types of bacteria have different properties,
- the protein themselves are different,
- the S-layers of the two are very different, and
- the secretion and anchoring mechanisms for each are different.

The skilled artisan would therefore not have considered that anything referred to in Smit *et al.* would be applicable to the S-proteins of the entirely different *Lactobacillus*.

35 U.S.C. 112 – Enablement

The Patent Office has the initial burden to question the enablement provided for the claimed invention. M.P.E.P. § 2164.04, and the cases cited therein. It is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. *In re Marzocchi*, 169 USPQ 367, 370 (C.C.P.A. 1971). Specific technical reasons are always required. See M.P.E.P. § 2164.04.

Claims 1-8 and 33-34 were rejected under Section 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants traverse.

Claim 1 is amended to clarify both the crystallization referred to is the ability to form a crystalline monolayer and the protein which is modified comes from a *Lacto-*

bacillus bacterium. It is submitted that Applicants' specification provides ample guidance to the skilled artisan to practice the claimed invention. In particular, the present specification describes how the skilled artisan can make, and assess, the modified *Lactobacilli* S-proteins claimed, including how to confirm that a particular modified S-protein can still form a crystalline monolayer, as specified by the pending claims.

As explained below, undue experimentation would not be required by the skilled artisan to practice the claimed invention. The attached Rule 312 Declaration by one of the inventors, Professor Pieter Pouwels, is now being submitted as evidence in this application. Each of the *Wands* factors listed in the Office Action are satisfied. Thus, the Applicants' specification enables the pending claims.

(i) Nature of the invention

Applicants agree with the assessment of the invention, although the fact that the claims have been limited from any S-protein to that of *Lactobacilli* needs to be taken into account as it means the claims are tightly based around what has been demonstrated in their specification.

It should be clarified, however, that the ability to crystallize, as specified by the claims, refers to the ability of the monomeric modified S-proteins to spontaneously form a two-dimensional crystalline monolayer, that, under natural circumstances, comprises the surface layer that envelopes the entire bacterial cell. Professor Pouwels outlines how that is the case in ¶ 5 of his Declaration:

> I think that a misunderstanding as to the meaning of the term "crystallisation" may have arisen contributing to the non-enablement rejection. In particular, reference in the claims to ability to crystallize, **refers to the ability of the modified protein to form a two-dimensional monolayer**, that, in a non-modified form, comprises the surface layer that envelopes the entire bacterial cell." [emphasis added]

Professor Pouwels goes on to highlight how such crystallization should not be confused with the three dimensional crystals formed in X ray crystallography, stating in ¶ 6 that:

> Thus, reference to a crystalline structure in the present instance **does not refer to the formation of the type of crystal that is used to determine the three-dimensional structure of a protein in X-ray crystallography**

> and the difficulties of generating such three dimensional crystals that is commonly associated with X-ray crystallography." [emphasis added]

As explained by Professor Pouwels, it appears that reference to crystallization in the claims has led to the incorrect idea that the claims refer to formation of the three-dimensional crystals used in X-ray crystallography. This is not correct. It refers to the natural property of the unmodified *Lactobacillus* S-proteins to form crystalline mono-layers. The claims are amended to emphasize this fact.

Professor Pouwels outlines how the skilled artisan would not interpret the claims as referring to three dimensional crystallization, stating in ¶ 7 of his Declaration that:

> Given that the specification deals with S proteins, **the ordinary reader would understand that is what is meant by reference to crystallisation** in the claims. [emphasis added]

Thus, any difficulties associated with forming crystals for X-ray crystallography have nothing to do with Applicants' claimed invention and should not give rise to a mistaken conclusion of nonenablement. This is a fundamental distinction which needs to be borne in mind when considering whether the present invention is enabled, because Applicants' specification does show the skilled artisan how to determine if a particular *Lactobacillus* modified protein can form a two-dimensional crystalline monolayer as specified by the claims.

(ii) Breadth of the claims

As amended, claim 1 is directed to an S-protein which originated from a *Lactobacillus*. The claims are therefore based around a single genus of bacteria and what is exemplified in the present application.

An important point to note is that reference to an S-layer protein is not the same as reference to any protein in the bacterial outer layers. Again, it appears that misinterpretation of the claims may have led the Examiner to believe that the claims are unduly broad in contrast to the reality that the claims are tightly based around one specific type of bacterial protein from *Lactobacillus*. As Professor Pouwels stated in ¶ 8 of his Rule 312 Declaration:

> The surface layer protein is defined by its ability to form a regular two-dimensional structure at the outside of a bacterium, composed of one sub-unit, the S-layer protein. An S-protein, thus, defines a protein present at the surface of bacteria that has the capacity to form a regular two-dimensional monolayer, either at the bacterial surface or in vitro . . .

Professor Pouwels then goes on to state that in ¶ 9 of his Rule 312 Declaration:

> The term S protein therefore defines a narrow subset of proteins with one S protein typically being found for each bacterium. **The term does not refer to any and all proteins found in the bacterial cell membrane or bacterial cell wall**. The present claims are not therefore directed to hugely broad array of proteins. They are directed to a **very specific, tightly related, protein type, fulfilling the same role in each bacterium**. That is particularly the case now the claims have been amended to refer to the protein coming from a *Lactobacillus*. [emphasis added]

Thus, the claims therefore involve a narrow class of proteins, typically with one protein per *Lactobacillus*, where the S proteins all share a common functionality and structure. Therefore, the pending claims are not unduly broad and the claim scope is reasonable.

(iii) Direction or guidance presented in the specification

The specification teaches the skilled artisan how to make and assess modified proteins to confirm that they retain the ability to crystallise. Again, confusion may have arisen between the generation of crystals for X ray crystallography and the specific two-dimensional monolayers that the modified S proteins can form and which are referred to in the claims. As Professor Pouwels stated in his Rule 312 Declaration's ¶ 11:

> Contrary to what is stated in the Official Action, the specification is not silent with regard to which bacterial surface protein will crystallise, or with regard to how to practice the claimed invention. On the contrary, **the specification describes the nature and properties of the modified *Lactobacillus* S-proteins and how to determine whether or not a modified S protein can form a crystalline monolayer** as specified by the claims. [emphasis added]

The Rule 312 Declaration goes on to outline in ¶ 12 how pages 44 to 60 of the present specification describe in detail how DNA sequences coding for *Lactobacillus* S-layer proteins with internal insertions encoding heterologous amino acid sequences can be constructed and how such sequences may be expressed in different bacterial species.

Thus, considerable guidance is provided. As Professor Pieter Pouwels stated in his Rule 312 Declaration's ¶ 13:

> Of particular relevance is the information on pages 45 and 46 of the specification, particularly Table 1 on page 45 of the specification. This shows that insertions at five different locations led to modified *Lactobacillus* S proteins that retained the capacity to crystallize (form a regular two-dimensional monolayer) as specified by the claims. The skilled artisan is **therefore able to identify those insertions for any given heterologous polypeptide that will result in a modified *Lactobacillus* S protein retaining the ability to form a crystalline monolayer**. [emphasis added]

Professor Pouwels then goes on to highlight that the Examples provide simple assays allowing the skilled artisan to ascertain whether or not any of the modified *Lactobacillus* S-proteins retain the ability to crystallize, stating in his Rule 312 Declaration's ¶ 14:

> The specification therefore describes a **simple assay**; the formation of a precipitate upon dialysis of hybrid S-proteins expressed in *E. coli*, which can be used to identify or confirm modified *Lactobacillus* S layer proteins retain the ability to form S layers. The specification also describes how electron microscopic analysis of the protein precipitates can be used to confirm ability to crystallize. [emphasis added]

Thus, the specification provides ample guidance to the skilled artisan of how to put the invention into practice, describing how to make and assess modified *Lactobacillus* S-proteins. The skilled artisan is left in no doubt as to how to do so and hence can readily practice the claimed invention without any undue experimentation being required.

(iv) Presence or absence of working examples

Applicants' specification does provide working examples of modified *Lactobacillus* S proteins still able to crystallize. As Professor Pieter Pouwels stated in his Rule 312 Declaration's ¶ 17:

> The Examples presented in the application show five different modified *Lactobacillus* surface layer proteins demonstrating the capacity to form regular two-dimensional crystalline monolayers. Working examples are therefore provided and the invention is reduced to practice.

The present specification does provide a number of different examples of the modified *Lactobacillus* S-proteins that retain the ability to form a crystalline monolayer. It is not the case that the present specification only provides a single example of a modified

protein, Applicants have provided many different examples and that should be taken into account. The limitation of the claims to modified *Lactobacillus* S-proteins should also be taken into account.

(v) State of the prior art

The Declaration also outlines how Bowie *et al.* does not provide any indication that the invention could not be put into practice. In particular, Bowie *et al.* is concerned with a wide variety of aspects of protein structure and in particular three-dimensional structure. As Professor Pieter Pouwels stated in his Rule 312 Declaration's ¶ 20:

> Bowie *et al* is concerned with whether insertion of foreign amino acid sequences in a protein will affect the proteins functional properties by changing the three dimensional structure of the protein and change its ability to form a three dimensional crystal of the type used in X ray crystallography. **That is not of relevance of the ability of the modified S protein to form a two dimensional crystalline monolayer as specified by the claims.** Bowie *et al* does not therefore cast any doubt on the ability of the invention to be put into practice. [emphasis added]

Thus, nothing in Bowie *et al.* should have an impact on the ability of the invention to be put into practice.

(vi) Quantity of experimentation necessary

As set forth above, the Examples of the present application provide a simple means to determine if a modified *Lactobacillus* surface layer protein falls within the scope of the claims: i.e., the ability to form a two-dimensional sheet, which is visible as a precipitate. Such experimentation is not undue and represents routine methodology well within the skill of the ordinary artisan.

This is emphasized in the Rule 312 Declaration's ¶ 21, where Professor Pieter Pouwels states:

> The **simple test** taught by the specification and Examples **does not represent undue experimentation** and the skilled artisan is readily able to both generate modified *Lactobacillus* S proteins and to assess their ability to form two-dimensional crystalline monolayers as specified by the claims. [emphasis added]

Thus, the skilled artisan needs no more than routine methodology to put the invention into practice and to assess whether or not a particular modified *Lactobacillus* S-protein retains the ability to form a two-dimensional monolayer.

(vii) Summary

Thus, the specification teaches the skilled artisan how to make and use modified S-proteins and to assess their ability to form two-dimensional crystalline monolayers. Specific working examples of such modified *Lactobacillus* S-proteins are provided. The skilled artisan person is therefore able to put the invention into practice without needing any undue experimentation. Applicant submit therefore that the claims are enabled.

Withdrawal of the enablement rejection made under Section 112, first paragraph, is requested because it would not require undue experimentation for a person of skill in the art to make and use the claimed invention.

35 U.S.C. 112 – Written Description

The specification must convey with reasonable clarity to persons skilled in the art that applicant was in possession of the claimed invention as of the filing date sought. See *Vas-Cath v. Mahurkar*, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). But the Patent Office has the initial burden of presenting evidence or a reason why persons of ordinary skill in the art would not have recognized such a description of the claimed invention in the original disclosure. See *In re Gosteli*, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

Claims 1-8 and 33-34 were rejected under Section 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants traverse. The present specification provides ample written description to illustrate that the present inventors were in full possession of the invention. As discussed above, the Examples of the specification provide representative examples of modified *Lactobacillus* S layer proteins that are able to form crystalline monolayers as required by the pending claims.

These issues are discussed in the attached Rule 312 Declaration of Professor Pouwels. In particular, in ¶ 24 of his Declaration, Professor Pouwels again emphasizes:

> S proteins from *Lactobacillus* bacteria represent a tightly defined group of proteins. The specific modified S proteins described in the specification do therefore provide a representative and adequate illustration of the invention. Furthermore, the specification describes the necessary tests to show that a given modified *Lactobacillus* S protein can form a crystalline monolayer as specified by the claims as also discussed above.

Professor Pouwels in his Declaration then goes on to outline in ¶¶ 25 and 26 how the present specification provides a number of illustrative examples which show that the invention is applicable to all *Lactobacillus* S-proteins based on the specific Examples taught. As stated in ¶ 26 of the Rule 312 Declaration:

> Moreover, the Example shows insertion of a heterologous peptide in **five different locations that retain the ability to form a two-dimensional crystalline structure** and hence fall within the defined group as indicated by the claims. These represent the "representative number of *Lactobacillus* S-proteins with inserted heterologous proteins that can still crystallize" referred to in he Official Action. [emphasis added]

As stated in ¶ 27 of the Declaration, the specific modified proteins generated do represent a demonstration that the invention is applicable to all *Lactobacillus* S-proteins as:

> It is reasonable to extrapolate from the results seen in the Examples to S proteins from Lactobacillus bacteria in general given that such S-proteins have generic properties.

In ¶ 27 of his Declaration, Professor Pouwels then goes on to state that such a generalization is reasonable since:

> All *Lactobacillus* S-proteins share a number of common characteristics which are not found in non S-layer proteins, the most prominent one being the capacity to form a regular two-dimensional array on the surface of bacteria or in vitro (in the absence of bacteria). **These special features allows the extrapolations from the S-protein of *L. acidophilus* dealt with in the Examples to other S-proteins from *Lactobacillus* bacteria to be made**." [emphasis added]

Thus, because of the common properties of all *Lactobacillus* S-proteins, the specific examples of modified proteins described in the specification do provide adequate written description for the claims under consideration.

Withdrawal of the written description rejection made under Section 112, first paragraph, is requested because the specification conveys to a person skilled in the art that Applicants were in possession of the claimed invention as of the filing date.

35 U.S.C. 112 – Definiteness

Claims 6-7 were rejected under Section 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants traverse. The attached Rule 312 Declaration also addresses the indefiniteness rejections and establishes that the terminology used in the claims would be well understood by skilled artisans.

In respect of the rejection of claim 6, Professor Pouwels highlights in ¶ 32 of his Declaration that:

> **the term "pI" is one frequently used in the art** and refers to the isoelectric point of a protein, i.e., the pH at which a protein has no net charge. Those working in the field would therefore understand what is meant by the term. [emphasis added]

Thus, the term "pI" is used in the art, would be well understood and does not therefore lead to any indefiniteness.

The Rule 312 Declaration also addresses the objection to the phrase "an antigen causing or specific for a disease" in claim 7. Professor Pouwels highlights in ¶ 33 of his Declaration that:

> the terminology would again be clear to the person in the field. In particular an antigen causing a disease is one responsible for the disease itself, such as an auto-antigen responsible for the development of an autoimmune disease. In contrast, an antigen specific for a disease is one, for instance, representative of a pathogen responsible for a disease. **Those working in the field would therefore be able to understand what is meant by the claims.** [emphasis added]

Thus, the phrase is not vague and indefinite, and would be well understood by the skilled artisan when the claims are read in light of the present specification.

Applicants request withdrawal of the Section 112, second paragraph, rejection because the pending claims are clear and definite.

Conclusion

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and

earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

Gary R. Tanigawa
Reg. No. 43,180

901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100